Bulletin Penelitian Kesehatan
Health Studies in Indonesia

Vol. II. No. 2
1974

THE USE OF THE INDIRECT HEMAGGLUTINATION TEST FOR THE DIAGNOSIS OF EXTRA – INTESTINAL AMEBIASIS IN JAKARTA

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Mikro indirek hemagglutinasi test dengan antigen axenik dari Entamoeba histolytica telah dipakai untuk mendapatkan zat2 anti amuba dalam sera dari 15 kasus abses hati yang pasti dan 4 kasus yang tidak pasti, 13 kasus disenteri amubawi akuta, 6 kasus colitis amubawi chronis, satu asymptomatik carrier, 39 pasien yang menderita penyakit2 lain dari 43 donor darah. Sera dari abses hati terdapat 100 per sen positif untuk zat2 anti amuba sedangkan sera dari orang2 dengan colitis amubawi chronis dan sera dari kasus disenteri amubawi akuta terdapat positif dalam urutan 50 dan 15 persen. Titer dari sera abses hati berkisar antara 1 : 128 dan 1 : 4096 dan titer dari sera amubiasis intestinalis yang positif adalah 1 : 128. Dari kedua kontrol grup tidak terdapat zat2 anti dengan titer lebih dari 1 : 64 (tabel 1 dan 2). Dari penyelidikan ini dapat diambil kesimpulan bahwa test indirek hemagglutinasi test dapat dipakai untuk occult invasive amubiasis bila metoda2 yang lazim gagal menemukan parasit.

In clinical amebiasis, the most reliable method for diagnosis is the recovery of the tissue-form of the *Entamoeba histolytica* from pathological specimens of patients. In amebiasis of the colon this traditional method gives almost good results; however in extra-intestinal amebiasis it is not often successful. The immunological approach of the problem on the other hand has given quite satisfying results and since the early investigations of Terry and Bozicevich (1949) and Hussey and Brown (1950) with the complement fixation test numerous serological studies had been made on amebiasis. From the various serological techniques developed during the course of time, such as the double diffusion principle of Ouchterlony (1948; 1958), the passive hemagglutination reaction (Boyden, 1951), the coated inert particle aggregation test (Ager et all, 1959. Christian et all, 1958), the immuno-fluorescent indirect method (Coons et all, 1941; Coons & Kaplan, 1950; Beutner et all, 1965), and immuno-electrophoresis (Grabar and Williams, 1955), two had found practical application for the routin serodiagnosis of amebiasis : The double diffusion test (Maddison, 1965, Maddison et all, 1965 (1) & (2); Powell et all, 1965; Powel et all, 1966) and the indirect hemagglutination test (Kessel et all, 1961; Kessel et all, 1965; Milgram et all, 1966; Maddison et all, 1968; Kasliwal et all, 1970). Both tests have been compared and evaluated by Maddison et all, (1965), (1) and Kagan & Norman (1970) and were found of equal sensitivity. The agar-gel-precipitin test has the advantage that it is simpler to perform, however it requires a concentrated antigen and an incubation period of at least 19–72 hrs and the test cannot be quantitated.

In this presentation the indirect hemagglutination test (IHA) is used for the serological studies of human amebiasis, in particular of the extra-intestinal amebiasis in Jakarta.

MATERIALS AND METHODS

Indirect hemagglutination test. The indirect hemagglutination test described by Kessel and Lewis (1961) and Lewis and Kessel (1961) was employed with minor modifications. A 2.5 per cent suspension of human red blood cells type 0 was tanned with tannic acid (Perla Merba, R.P.) at 1 : 20.000 dilution in a 37°C waterbath for 15 minutes. The tanned cells were sensitized with antigen at the proper dilution at a pH of 6.4 ± 0.2 in a 37° C waterbath for 15 minutes and used immediately after washing. The tests were performed with the microtiter equipment purchased from Flow Laboratories, Rockville, Maryland. Sera were diluted serially from 1 : 2 to 1 : 4096 dilution and the tests were read after an incubation of 30 minutes at room temperature and then 18 hr at 5 – 10°C. A negative reaction showed

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a tiny button or a smooth ring of sedimented cells at the bottom of the well, a positive reaction showed a carpet of agglutinated cells with irregular edges. Only strong positive reactions were read. Sera which gave titers lower than 1 : 128 were recorded as negative. The antigen employed was a lyophilized antigen obtained from Parke & Davis and from the Parasitological Unit of C.D.C. Atlanta, Georgia. In the hemagglutination test the Parke & Davis antigen was used at 1 : 8 dilution and the C.D.C. antigen at 1 : 10 dilution. The dilution of each brand of antigen was determined against serial dilutions of two high positive (sera with titers greater than 1 : 256), two low positive (sera with titers 1 : 128 or 1 : 256), and two negative reference sera. These reference sera were obtained from C.D.C. Atlanta.

A. Sera. 78 patients from Dr Tjipto Mangunkusumo, Persahabatan, Cikini hospitals and from a few patients treated ambulatory at the Department of Parasitology were studied. The age of the patients ranged from 18 to 60 years.

These patients were grouped according with their clinical records into the following :

Confirmed amebic liver abscess. Fifteen patients with amebic liver abscess, confirmed by aspiration of typical pus or demonstrating *E. histolityca* trophozoites in pus.

Unconfirmed amebic liver abscess. Four patients in whom a clinical diagnosis of liver abscess was made without aspiration of pus.

Acute intestinal amebiasis. Thirteen patients with amebic dysentery, confirmed by the presence of hematophagous trophozoites in the stools.

Chronic intestinal amebiasis. Six patients in whom amebic colitis developed several months following amebic diarrhea.

Asymptomatic carrier. One person who came to the Department for health certificate harbouring *E. histolytica* cysts in the stools.

Other diseases. Thirty-nine patients suffering from other diseases such as : 9 liver diseases, carcinoma of liver, cirrhosis of liver, pyogenic liver abscess, hepato-splenomegaly, 23 lung diseases such as pulmonary tuberculosis, tuberculous pleural effucion, bacterial empyema, pyogenic lung abscess, carcinoma of lung, 3 cases of nephritis, 1 Hodgekin's disease, 1 typhoid fever, 2 cases of undetermined dysentery.

B. Sera from 43 blood donors which were obtained from the Indonesian Red Cross Clinic at Dr. Tjipto Mangunkusumo hospital. The age of the donors ranged from 18 to 50 years and most of them were males. All sera were handled with a-septic precautions and were stored at 20°C. They were examined for the indirect hemagglutination test in groups of 25 sera at a time.

RESULTS

Table 1 shows the results of the IHA with 78 sera from various disease groups and with 43 sera from blood donors.

Table 1. Results of IHA on sera from amebiasis, other diseases and blood donors

Disease group and blood donors	Total number examined	Negative	Positive		percentage No. positive/total No. examined
		No agglutination or agglutination with titer lower than 1 : 128	Agglutination titer 1 : 128	Agglutination titer 1 : 256 or higher	
1. Confirmed amebic liver abscess	15	0	2	13	100
2. unconfirmed amebic liver abscess	4	2	0	2	50

3. acute intestinal amebiasis	13	11	2	0	15
4. chronic intestinal amebiasis	6	3	3	0	50
5. asymptomatic carrier	1	1	0	0	0
6. Other diseases	39	39	0	0	0
7. Blood donors	43	43	0	0	0

Liver abscesses. All sera from 17 cases of confirmed amebic liver abscesses and two sera from unconfirmed amebic liver abscesses were positive for amebic antibodies. Most of these sera titers were high, up to 1 : 4096 and only two sera had 1 : 128 titer.

Intestinal amebiasis. The results of the IHA on 20 selected cases of intestinal amebiasis showed a definite higher frequency of amebic antibodies with high titer (1 : 128) in the chronic stage than in the acute cases whilst one asymptomatic carrier had no antibodies. Low antibody titers were found in 3 cases of acute intestinal amebiasis (table 2).

Table 2. Low titer amebic antibodies found in the indirect Hemagglutination test.

Clinical state	Number of cases	hemagglutination titer		
		1 : 16	1 : 32	1 : 64
Acute intestinal amebiasis	3	2	–	1
lung abscesses with & without hepatitis	4	1	–	3
blood donors	11	2	4	5

Other diseases and blood donors. In these groups no serum has antibodies up to 1 : 128 dilution of serum. Amebic antibodies of low titers were found in four out of 39 persons suffering from other diseases and in 11 blood donors. The frequency distribution of these low titer serums are shown in table 2.

DISCUSSION

The finding of 100 per cent frequency for amebic antibodies with high titers in amebic liver abscess proves a high sensitivity of the IHA for the diagnosis of extra-intestinal amebiasis and emphasizes its value in detecting occult invasive amebiasis. These results are in conformity with those reported by various workers such as Kessel et all (1961), Kasliwal et all (1970), who found a 100 per cent frequency of positive test in liver amebiasis and Milgram et all (1966) who found a 99 per cent frequency.

The results of the test on intestinal amebiasis however are markedly different from those of Kessel et all (1961), Milgram et all (1966), Healy (1968) and Krupp (1970). Kessel et all (1961) found a 98 per cent frequency of amebic antibodies in 37 sera from intestinal amebiasis whereas Milgram et all (1966), Healy (1968) and Krupp (1970) found respectively 82, 85 and 81 per cent frequency in their material of respectively 83, 63 and 168 cases of amebic dysentery. In this study a 26 per cent positive test was found in 19 cases of symptomatic intestinal amebiasis. In a similar small number of material (18 cases) Kasliwal and

Associates (1970) found a higher (44.4) percentage of positive test. It should be mentioned, however, that these last authors used the 1 : 16 titer as the diagnostic titer for advanced amebiasis. Many factors play a role in inducing antibodies in intestinal amebiasis, such as the extend of the lesions in the intestinal mucosa caused by the invasion of the amebia, the response of the host as well as the time at which the blood sample is drawn. When the serum sample is drawn only a few days after the elicit of symptoms, the change of getting a positive result is meagre. This fact can be shown from the frequency of the positive test found in the chronic forms of intestinal amebiasis. Other factors which may cause discrepancies in results are the use of different techniques, such as differences in the kind of red blood cells, differences in the use of positive reference sera, and differences in interpretation of positive tests from the titers given by the sera. It is known that sheep red blood cells are more sensitive for tanning procedures than human red blood cells and thus it might be possible that the liver abscess sera in this study gave much lower titers than those in the studies of Milgram et all (1966) who reported to find and average titer of 1 : 12000 in sera from liver abscesses.

The interpretation of a positive IHA from a given titer has been of much dispute in the past. Kessel et all (1961) compared the hemagglutination test with the complement fixation test and found no agreement of positive hemagglutination test between 1 : 8 – 1 : 32 and partial agreement from 1 : 128 and higher. Maddison (1965) and many others were of the opinion that the hemagglutination test is a more sensitive test than the agar-gel-precipitin test whilst the complement fixation test is the least sensitive test for amebiasis. Because of its sensitivity the recognition of a positive test from the titer is important for the hemagglutination test. Milgram et all (1966) regarded a titer of 1 : 128 as the lowest positive titer. Investigating a large number of selected groups of cases of amebiasis and using several selected groups of controls, they found good correlation between a positive hemagglutination test and clinical amebiasis. Similarly, Healy (1968; 1970) using the same technique as Milgram et all had found high specificity and sensitivity of the hemagglutination test. A method for evaluation of the hemagglutination test for clinical amebiasis had been presented by Krupp (1970). The sera and clinical state of 392 persons living in an endemic country were studied and the titers obtained in the IHA were plotted in a graph against frequency of distribution. Thus doing, a bimodal curve was obtained, showing one sharp (negative population) and one low (positive population) peak. By interpolating at the point of sharp decrease in percentage of persons at the negative curve a titer of 1 : 40 was found which then was taken as the highest negative titer. Thus the IHA applied to that particular population was established as being positive starting at 1 : 80 titer. Kasliwal and Associates (1970) in their studies of the significance of the IHA for the diagnosis of amebiasis in Jaipur, India, approached the problem of determination of the diagnostic titer of the IHA, by investigating sera from persons with clinical amebic hepatitis. They found in 16 sera (64%) antibodies with titers varying between 1 : 16 and 1 : 64. In two cases of more advanced invasive amebiasis with involvement of the lungs and pleura the antibody titer found was 1 : 16. Based upon above information the 1 : 16 titer was regarded as the lowest positive titer. In this preliminary study, the interpretation of the titer of a positive IHA was made by the study of the test on 15 sera from patients with frank amebic liver abscess. As shown in table 1 most of the sera had high titers up to 1 : 4096, but two had 1 : 128 titer. Consequently I have regarded the 1 : 128 titer as the lowest positive titer. Thus regarding the test as positive when hemagglutination was observed at 1 : 128 serum dilution, the majority (85%) of the sera from acute intestinal amebiasis were found negative whilst 50 per cent of the persons with a history of chronic amebic dysentery showed a positive test. A thorough clinical follow up study on these patients unfortunately had not been made so that it is not possible at this time to make any speculation as to whether these high anti-

body titers came from advanced processes in the intestinal wall or from other organs.

The significance of antibodies in serum dilutions lower than 1 : 128 cannot be evaluated in this study since the medical records on the patients with acute amebic dysentery did not include thorough clinical history, physical examination and routin laboratory investigations. As regards to the 4 patients with low titer antibodies in the group with other diseases, one had a hydrothorax and amebic antibody titer 1 : 16, 3 other patients with 1 : 64 titer had ulcerative lung abscesses of tuberculous origin. The clinical state of the blood donors from which blood samples were taken several months before the test was done, was unknown.

SUMMARY

The incidence of amebic antibodies as revealed by the indirect hemagglutination test was determined in 5 different groups of amebiasis, other patients with other diseases and in blood donors.

A 100 per cent correlation was found between the prevalence of antibodies of high titer (1 : 128 – 1 : 4096) and incidence of amebic liver abscesses. A higher incidence of antibodies of titer 1 : 128 was found in chronic than ir acute amebiasis of the colon.

None of the persons in the control groups had antibodies of significant titer. The above observations indicate that the indirect hemagglutination test is useful for the diagnosis of occult invasive amebiasis when the usual methods fail to show the organism.

ACKNOWLEDGEMENTS

I am very greatful to the Director of the National Institute for Medical Research for the funds given for this study.

I thank Dr S. Alisah Naoemar of the Department of Parasitology for many of the blood samples and clinical data from amebiasis patients, and Dr Kabul N. Idris for the blood samples from patients with other diseases. I am indebted to Professor Dr Bintari Rukmono for reading the paper.

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